



11/24/2023

**2023.11.24 Project Skunkworks: Radegen DNA Assembly™ T5-SfiI™**

The first core competency of Radegen Biotechnology is cloning reagents. Radegen DNA Assembly™ T5-SfiI™ is a purified protein reagent composed of T5 exonuclease, a high fidelity DNA ligase like Taq or Tth ligase, a proofreading polymerase like Psp polymerase and SfiI endonuclease. This system is a next-generation Golden Gate system and is characterized as so because it recombines multiple donor plasmids by first existing a dsDNA donor fragment from a plasmid and clones the fragment into a SfiI domesticated plasmid. The proofreading polymerase in the enzyme cocktail removes terminal end mismatch nucleotides and the T5 exonuclease removes any 5' mismatches while producing a 3' overhang. In a properly designed donor DNA with homology arms targeting the dsDNA fragment to a destination plasmid is said to have long homology sticky end overhangs. The enzymes in this system are chosen for an iso-thermal one pot DNA assembly reaction at 50 degrees celsius and the final product from this reaction consists of a seamless DNA construct. The revolutionary aspect of this system is the 8 bp rare cutter endonuclease, SfiI which minimizes the need to domesticate a destination plasmid and donor DNA from the common 1 in 4<sup>6</sup> or the less frequent 1 in 4<sup>7</sup> binding frequencies to 1 in 4<sup>8</sup> frequency of SfiI. Creative Commons licensing protects the use of T5 in combination with an endonuclease in any iteration. The T5-SfiI combination of enzymes is a purified protein version of T5-NotI and T5-SapI that is amenable to incubation at 50 degrees Celsius, facilitating an efficient one pot reaction with the other components of the system.

## &gt;T5 Exonuclease

MSKSWGKFIEEEAEMASRRNLMIVDGTNLGFRFKHNSKKPFASSYVSTIQSLAKSYSARTTIVLGDGKGSVFRLEHLPEYKGNRDEKYAQRTEEEKALDEQFEYLKDAFELCKTTFTPTFTIRGVEADDMAAIVKLIHLYDHWLISDGDGW  
DTLLTDKVSRSFSTTRREYHLRDMYEHNNVDVEQFISLKAIMGDLGDNIRGVEGIGAKRGYNIIEFRGNVLDIIDQLPLPGKQKIQNLNASEELLFRNLILVDLPCTVCDAIAAVGQDVLKDKFTKDILEIAEQ\*

## &gt;SfiI

MHQDYRELSDELESVEKQTLRTIVQALQQYSKEAKSIFETTAADSSGEVIVLAEDITQYALEVAETYPINRRAGFIDYKRVRLPSPHGLLPQVLLVDKASTEKNRDLQRSQPLMDAEFRNTSSGEVVTMEAGVIPHMLQSANDGVLPAVT  
TSIFVHYREYRLKDVGRYRLKSIYVLSLPHARKLQRYNPDPDTSFFGAGKHSAPGEARVIRVYDRLKEACPWRQLHYSDASEYTPQRWRDLNDAGHEVTKEFLFLER\*

## &gt;SfiI.M1

MRKPSSGQAGLDAWDETSLATHAGPDFALYVGDSDCLAKLPDESINTVVTSPPYWAVRDYEHDEQLGLEDEVDYVERLVKIFREYVRVLATDGSAWLNIGDSYFNKQITVGGKPPRTGWKRKQLSLVPFRVALAQDDGWWIRNVAVWHKP  
NAMPASVRDLRTVTWEPVFLTTSKERYYNLDEIRVPHQTSDAIERRAESGTVTGKAQKKELRKWLNSPRHRTIEGIEKVERRRPNAPAAVELASYLRTALKEKRSIAWVAEQDLDPFERTHRHYFRTDEIGSRLLPPEVMEQLKDLLELDATY  
DEAMTVEVGDNVFRNHPNGKNPGDLLSIPTAPSGANHFVAMPKRLAHFALKATLPMNGSCLDPFMGSGTGRVVRLEGGRFVGDVNEHYMTDYLVESGVISPETETLW\*

## &gt;taq ligase

MTLEAARRRVNELRDLIRYHNYLYVVDLDAPEISDAEYDRLLRELKELEERFPELQSPDPTQVGARPLESTFRPVRHPRMYSLDNAFSLDEVRAFEERIERALGRKGPLYTVEHKVDGLSVNLYEEGILVFGATRGDGETGEEVTQNLTLIR  
TIPRRLTGVPDRLEVRGEVYMPIEAFRLNQELEEAGERIFKNPRNAAAGSLRQKDPRTVARRGLRATFYALGLGLEETKQSHDLLLWLRERGFVVEHGFTRALGAEGVEEVYQAWLKERRLPFEADGVVVKLDDLALWRELGYTARAPFAL  
AYKFPAAEEKETRLLSVAFQVGRITPTVGVLEPVFIEGSEVSRVTLHNSYIEELDVRIGDWMVLVHKAGGVPEVLRLVKERRTGEEKPLMPENCPECGHALKEGKVHRCNPPLCPAKRFEAIRHYASRKAMDIOGLGEKLEKLLKGLVRD  
VADLYRLKKEDLVNLERMGKSAENLRQIEESKGRGLERLLYALGLPGVGEVLARNLALRFHGMDRLEAGLEDLLEVEVGELTARAILNTLKDFEPRDLVRLKEAGVMEAKEGEREGALKGLTFVITGELSRPREVKALLRRLGAKVTSV  
SRKTGFLVVGENPGSKLEKARALGVPTLSEELLYRLTEERTGKDPALTA\*

## &gt;tth ligase

MTLEEARKRVNELRDLIRYHNYRYVVDLDAPEISDAEYDRLLRELKELEERFPELQSPDPTQVGARPLEATFRPVRHPRMYSLDNAFNLDELKAFAFEERIERALGRKGPFAYTVEHKVDGLSVNLYEEGVLVYGATRGDGEVGEVTQNLTLIP  
TIPRRLKGVPERLEVRGEVYMPIEAFRLNQELEEAGERIFKNPRNAAAGSLRQKDPRTVARRGLRATFYALGLGLEEVEGATVQFALLHMLKEGKGFVVEHGYARAVGAEGVEAVYQDWLKKRRALPFEADGVVVKLDELALWRELGYTARAPR  
FAIAYKFPAAEEKETRLLDVVFGVGRITPTVGVLEPVFIEGSEVSRVTLHNSYIEELDVRIGDWMVLVHKAGGVPEVLRLVKERRTGEEKPLMPENCPECGHALKEGKVHRCNPPLCPAKRFEAIRHYASRKAMDIOGLGEKLEKLLKGLVRD  
VKDVADLYRLKKEDLVNLERMGKSAENLRQIEESKGRGLERLLYALGLPGVGEVLARNLALRFHGMDRLEAGLEDLLEVEVGELTARAILNTLKDFEPRDLVRLKEAGVMEAKEGEREGALKGLTFVITGELSRPREVKALLRRLGAKVT  
DSVSRKTSYLVVGENPGSKLEKARALGVPTLSEELLYRLTEERTGKKAELV\*

## &gt;psp polymerase

MIIDADYITDGGPIIRIFKKEKGEFVYDRTFRPYIYALLKDDSAIDEVKKITAERHGKIVRITVEKVQKKFLGRPIEVWKLYLEHPQDVPAIREKIREHPAVVDIFEYDIPFAKRYLIDKGLTPMEGNEELTFVLADITLYHEGEEFGKGP  
IIMISYADEEGAKVITWKSIDLPPYEVVSSERMEIKRLVKVIREKDPDVIITYNGDNDFPYLLKRAELGKIKLPLGRDNSEPKMQRMGDSLAVEIKGRIHFDLPFAIRRTINLPTTYLTVEYVIFGKSKEKVYAEIAEAMETGKGLERVAKYS  
MEDAKVTSSELGKEFFPMEAQLARLVGHPVMDVSRSTGNLVEWFLTKAYERNELAPNPKDEREYERRLRSEYEGGYVNEPEKLGWEGIVSLDFRSLYPSIIITHNVSPDTLNRENCKEYDAPQVGHRCCKDFPGFIPSLNLNLEERQKIKKRM  
KESKQVEKLLDYQRRAIKILANSYGYGYAKARWYCKEACESVTAWGRQYIDLVRRELSRGFKVLYIDTDLGYATIPGAKHEEIKKALKFVEYINSKLPGLLEVEYGFYARGFFVTKKKYLIDEEGKIVTRGLEIVRRDWEIAKETQA  
KVLKAILKHGNDVAVKIVKVEKLSKYIEPPEKLYIEQITRPLSEYKAGPHVAVAKLAAGKVKVPGMVGIVLKGDPISKRAIAIEEFDPKKHKYDAEYIENQVLPAVERILRAFGYRKEDLRQYKTKQVGLGAWLKF\*